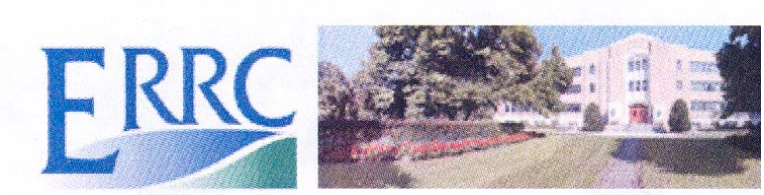

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EFFICACY OF ACIDIC ELECTROLYZED WATER AND OTHER SANITIZING SOLUTIONS IN REDUCING *ESCHERICHIA COLI* O157:H7 POPULATIONS IN INACCESSIBLE REGIONS OF THE APPLE

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ABSTRACT

This study was designed to show that attachment of pathogens to inaccessible sites (calyx and/or stem) of the apple is a major factor in limiting the efficacy of washing treatments. Apples that were artificially contaminated with *Escherichia coli* O157:H7 in inaccessible regions were washed at 25 and 60°C for 2 min with shaking in one of the following washing solutions (4 L): tap water, hydrogen peroxide (5%); acidified sodium chlorite (Sanova; 1200 ppm); chlorine (400 ppm; pH=6.5); acidic electrolyzed water (AEW; 30 ppm chlorine; pH=2.4). Overall, none of the washing treatments used was able to completely inactivate and/or remove pathogenic cells attached to inaccessible sites of the apple. The failure of these washing treatments to inactivate and/or remove bacterial cells in inaccessible sites demonstrates the need for new fruit washing technology that can overcome this limitation.

INTRODUCTION

Fresh fruits and vegetables, including unpasteurized fruits juices, have been established as vectors for foodborne illness. Several outbreaks of foodborne disease associated with *Escherichia coli* O157:H7 (*2,3,5,6*), *Salmonella* (*4*), and *Cryptosporidium* (*6*) in unpasteurized apple cider have been reported. The fact that this is a ready-to-eat product, receiving no further processing before consumption, is a matter of concern. These outbreaks have prompted the Food and Drug Administration, to mandate that all cider producers to have HACCP programs in place and that fresh juice product be treated with a process designed to yield a 5-log reduction in the most resistant organisms of public concern (*7*). The presence of pathogens on the surface of apple also has implications for safety applicable to the fresh and fresh-cut fruit markets. A recent recall of commercial fresh-cut apples contaminated with *Listeria monocytogenes* raised concerns about the microbiological safety of fresh-cut apple products (*8*). Laboratory washing studies of apples, using water, detergents or sanitizing agents, were reported to produce up to 3-log reduction in the levels of *E. coli* (*9,10*). However, when these same treatments were applied using a commercial flatbed brush washer, there was less than 1-log reduction in *E. coli* populations (*1*). Survival of bacteria during washing treatments was attributed to the attachment of *E. coli* cells to inaccessible regions in the stem and calyx areas of apples, and possible infiltration into the calyx channel and the core of the apple (*1,10*).

EFFICACY OF WASHING TREATMENTS IN REDUCING *E. COLI* O157:H7 POPULATIONS INSIDE THE CALYX OF THE APPLE

The effects of washing treatments on population reduction of *E. coli* O157:H7, spot inoculated inside the calyx cavities of apples, are shown in Table 1. Inoculum level on control (untreated) apple samples was ca. 5.2–6.7 log CFU *E. coli* O157:H7/gm (Table 1). Washing treatments were performed at 25 or 60°C for 2 min with shaking. Heating apples at 60°C for 2 min had no adverse effect on the color and firmness qualities of the apple, as compared to treatments at higher temperatures or longer exposure time (>2 min) at 60°C. Data presented here showed that none of the washing treatments used differed significantly or was effective in inactivating or removing *E. coli* O157:H7 cells attached to the calyx region of the apple (Table 1). This can be attributed to the poor contact between the washing agent and bacterial cells artificially attached to the calyx (inaccessible site). Therefore, efficacy of the washing treatment is limited by the attachment of the bacterial cells to inaccessible areas of the apple.

EFFICACY OF WASHING TREATMENTS IN REDUCING *E. COLI* O157:H7 POPULATIONS IN THE STEM OF THE APPLE

The effects of washing conditions on population reduction of *E. coli* O157:H7, spot inoculated at the base of stems of apples, are shown in Table 2. Control (untreated) apple samples were inoculated with ca. 5.2-6.5 log CFU *E. coli* O157:H7/gm (Table 2). There was up to 2 log reduction in *E. coli* O157:H7 populations using 1200 ppm Sanova at 25°C (Table 2). Population reductions resulting from washing with water and acidic electrolyzed water were significantly lower ($p<0.05$) than population reductions obtained with other washing agents (Table 2). Data presented here showed that none of the washing treatments was able to completely inactivate and/or remove *E. coli* O157:H7 cells artificially attached to the stem region of the apple. This could be attributed to poor contact between the washing solution and bacterial cells in this inaccessible site of the apple. Therefore, efficacy of the washing treatment can be limited by the attachment of bacterial cells to the stem area of the apple. This is similar to the results obtained with washing treatments of artificially inoculated apples in the calyx region (see above).

EFFICACY OF WASHING TREATMENTS IN REDUCING *E. COLI* O157:H7 POPULATIONS ONTO THE SKIN OF THE APPLE

The effects of washing treatments on population reduction of *E. coli* O157:H7, spot inoculated on skin sites of apples, are shown in Table 3. The inoculated control (untreated) showed a population of ca. 4.7-6.44 log CFU *E. coli* O157:H7 /gm apple (Table 3). The increase in washing temperature did not correspond to a significant increase in reducing *E. coli* O157:H7 populations on the skin of apples (Table 3). Population reductions obtained with water were similar to reductions obtained with sanitizers. These reductions were significantly higher than those obtained with apples artificially inoculated inside the calyx (Table 1) or at the base of the stem (Table 2). These larger reductions can be attributed to a better contact between the washing agents and bacterial cells on the skin of the apple. The incomplete inactivation and/or removal

of *E. coli* O157:H7 cells on the skin of the apple could be attributed to: 1) short exposure time (2 min) to washing agents, and/or 2) biofilm formation on the surface of the apple.

CONCLUSIONS

♦ Major factors that are important in limiting the efficacy of washing treatments of apples are the attachment of bacterial cells to inaccessible sites (calyx, and stem) of the apple, and the incorporation of those cells within biofilm in inaccessible sites of the apple. New technology that improves the contact between inaccessible bacterial cells and an antimicrobial wash, accomplishes pasteurization, or physically removes these pathogenic organisms from calyx, stem, and/or biofilm, is required.

♦ Washing treatments at 60°C were no more effective than treatments at 25°C in reducing *E. coli* O157:H7 populations (Tables 1-3). Although, in principle thermal treatments would seem to be a potential apple process, it has adverse effects on apple quality. Therefore, further research is required for developing alternative surface treatments effective against pathogens attached to inaccessible sites of apples intended for fresh and/or fresh-cut markets.

♦ Although washing treatments using water were not significantly different from washing with sanitizing agents, it is recommended that sanitizing agents be used during all washing treatments of apples. The use of a sanitizing agent during washing treatments of apples would reduce the microbial load in the washing solution and thus prevent any possible cross contamination in the washing tank.

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Table 1. Effect of washing treatments on population reduction[1] of *Escherichia coli* O157:H7 applied to the calyx region of the apple.

Washing Solution	Inoculated Control[2] (log_{10} CFU/gm)	Population Reduction (log CFU/gm)	
		25°C	60°C
Tap Water	6.71	0.19 ± 0.18 AB	0.43 ± 0.15 AB
5% Hydrogen Peroxide	5.64	0.39 ± 0.08 AB	0.80 ± 0.44 AB
1200 ppm Sanova[3]	5.80	0.48 ± 0.09 AB	1.06 ± 0.14 A
400 ppm Chlorine (pH[4] = 6.5)	6.11	0.66 ± 0.37 AB	0.95 ± 0.28 A
Acidified Electrolyzed Water	5.18	-0.04[5] ± 0.20 B	-0.09[5] ± 0.28 B

[1] Mean of cell population (duplicate samples) following washing treatment minus means of cell population (duplicate samples) of untreated inoculated control. Means with no letter in common are significantly different at $p<0.05$.

[2] Mean cell population of duplicate untreated inoculated samples.

[3] Sanova (acidified sodium chlorite) solution was prepared according to the manufacturer's specifications.

[4] The pH of the chlorine solution was adjusted to 6.5 using concentrated hydrochloric acid.

[5] Negative numbers indicate no reduction in cell populations was detected following washing treatment.

Table 2. Effect of washing treatment on population reduction[1] of *Escherichia coli* O157:H7 applied to the stem region of the apple.

Washing Solution	Inoculated Control[2] (log_{10} CFU/gm)	Population Reduction (log_{10} CFU/gm	
		25°C	60°C
Tap Water	6.37	-0.10[3] ± 0.12 D	0.11 ± 0.0.12 D
5% Hydrogen Peroxide	5.50	1.83 ± 0.17 AB	0.96 ± 0.72 BC
1200 ppm Sanova[4]	5.66	2.24 ± 0.68 A	2.04 ± 0.62 AB
400 ppm Chlorine (pH[5] = 6.5)	6.53	0.49 ± 0.51 CD	1.56 ± 0.26 ABC
Acidified Electrolyzed Water	5.19	-0.20[3] ± 0.27 D	-0.30[3] ± 0.30 D

[1] Mean cell population (duplicate samples) following washing treatment minus mean cell population (duplicate samples) of untreated inoculated control. Means with no letter in common are significantly different at $p<0.05$.
[2] Mean cell population of duplicate untreated inoculated samples.
[3] Negative numbers indicate no reduction was detected following washing treatment.
[4] Sanova (acidified sodium chlorite) solution was prepared according to the manufacturer's specifications.
[5] The pH of the chlorine solution was adjusted to 6.5 using concentrated hydrochloric acid.

Table 3. Effect of washing treatment on population reduction[1] of *Escherichia coli* O157:H7 applied to the skin region of the apple.

Washing Solution	Inoculated Control[2] (log_{10} CFU/gm)	Population Reduction (log_{10} CFU/gm)	
		25°C	60°C
Tap Water	6.37	3.71 ± 0.25 AB	4.23 ± 1.24 AB
5% Hydrogen Peroxide	5.24	3.97 ± 1.20 AB	3.74 ± 0.68 AB
1200 ppm Sanova[3]	5.49	4.38 ± 0.45 AB	4.83 ± 0.75 A
400 ppm Chlorine (pH[4] = 6.5)	5.39	3.00 ± 1.23 ABC	4.84 ± 0.15 A
Acidified Electrolyzed Water	4.65	1.64 ± 0.19 C	4.07 ± 0.37 AB

[1] Mean cell population following (duplicate samples) washing treatment minus mean cell population (duplicate samples) of untreated inoculated control. Means with no letter in common are significantly different at $p<0.05$.
[2] Mean cell population of duplicate untreated inoculated samples.
[3] Sanova (acidified sodium chlorite) solution was prepared according to the manufacturer's specifications.
[4] pH of the chlorine solution was adjusted to 6.5 using concentrated hydrochloric acid.